# Nutritional Status Changes in Humans during a 14-Day Saturation Dive: The NASA Extreme Environment Mission Operations V Project[1,2]

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ABSTRACT Ground-based analogs of spaceflight are an important means of studying physiologic and nutritional changes associated with space travel, and the NASA Extreme Environment Mission Operations V (NEEMO) is such an analog. To determine whether saturation diving has nutrition-related effects similar to those of spaceflight, we conducted a clinical nutritional assessment of the NEEMO crew (4 men, 2 women) before, during, and after their 14-d saturation dive. Blood and urine samples were collected before, during, and after the dive. The foods consumed by the crew were typical of the spaceflight food system. A number of physiologic changes were observed, during and after the dive, that are also commonly observed during spaceflight. Hemoglobin and hematocrit were lower ($P < 0.05$) after the dive. Transferrin receptors were significantly lower immediately after the dive. Serum ferritin increased significantly during the dive. There was also evidence indicating that oxidative damage and stress increased during the dive. Glutathione peroxidase and superoxide dismutase decreased during and after the dive ($P < 0.05$). Decreased leptin during the dive ($P < 0.05$) may have been related to the increased stress. Subjects had decreased energy intake and weight loss during the dive, similar to what is observed during spaceflight. Together, these similarities to spaceflight provide a model to use in further defining the physiologic effects of spaceflight and investigating potential countermeasures. J. Nutr. 134: 1765–1771, 2004.

KEY WORDS: • *saturation diving* • *hyperbaric* • *nutrition* • *spaceflight analog*

Nutrition is essential for the maintenance of crew health before, during, and after spaceflight. Several physiologic changes occur during spaceflight, including bone and muscle loss (1), oxidative damage (2), and cardiovascular and hematologic changes (3). These may involve some degree of altered nutritional status. Ground-based models have been used extensively to study human adaptation to spaceflight (4). They include disuse (e.g., bed rest) and isolation (e.g., Antarctic and closed-chamber studies). Underwater analogs have also been used to simulate the isolation, stress, and constraints of spaceflight. They are used to better understand the physiologic and psychologic effects of such environments on humans, to study training and operational issues, to evaluate hardware and procedures, and to test the effectiveness of potential countermeasures.

In an underwater-based analog called the NASA Extreme Environment Mission Operations (NEEMO)[4] project, subjects live in an underwater habitat for extended periods. The unique underwater laboratory, Aquarius, provides an environment similar to that aboard the International Space Station (ISS). Not only is the habitat similar in size to modules of the ISS, but the "aquanauts" coordinate operations remotely via a mission control center located onshore (4.5 km away). The food system, as in the study reported here, may mimic that of ISS astronauts, and crew members also perform extensive scientific and extravehicular activities during missions.

The environment in the habitat emulates that of the ISS, as shown by the stress-induced physiologic changes that are also commonly observed during spaceflight (5) and in other ground-based analogs (6). Some of the physiologic changes that occur during spaceflight are thought to result from increased stress caused by environmental changes such as acceleration during lift-off, weightlessness, confinement, and long-term maintenance of high levels of performance. These types of stress induce hormonal changes and altered immune func-

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[4] Abbreviations used: 8(OH)dG, 8-hydroxy-2′-deoxyguanosine; D, dive minus day; DEXA, dual energy X-ray absorptiometry; DPD, deoxypyridinoline; GPX, glutathione peroxidase; ICP-MS, inductively-coupled plasma MS; ISS, International Space Station; LBNP, lower body negative pressure; MD, mission day; MDA, malondialdehyde; NEEMO, NASA Extreme Environment Mission Operations; 25-OH vitamin D, 25-hydroxy vitamin D; 1,25-$(OH)_2$ vitamin D, 1,25-dihydroxy vitamin D; PYD, pyridinium crosslinks; R, return day; SOD, superoxide dismutase.

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tion (7–9). Although these stress-induced changes occur during spaceflight, the confounding effects of altered nutritional status during flight (and the effects of stress on nutritional status) are not well understood and should be clarified to define nutritional requirements for long-term spaceflight.

The aim of this study was to evaluate the nutritional status of subjects in a ground-based analog of spaceflight, the 5th NEEMO mission (NEEMO V). A comprehensive nutritional assessment was conducted before, during, and after the mission. We hypothesized that in addition to the effects of stress and confinement, the unique characteristics (such as increased atmospheric pressure) of the mission and habitat would also affect nutrition and health.

## SUBJECTS AND METHODS

***Environment.*** NEEMO V was a 14-d saturation dive, with the divers ($n = 6$) living in an underwater habitat. The habitat is 14 m long and 4 m in diameter (**Fig. 1**). It is located 19 m (62 ft) below the ocean surface, and the atmospheric pressure inside the habitat is 253 kPa (2.5 atm). The NEEMO V mission took place in June-July of 2003. Supplies were transferred down to the habitat via sealed container, and samples were returned to the surface via the same container. All tubes and hardware were pretested to ensure that the pressure change would not alter function. Because of the nature of the dive (an extended-duration saturation dive), divers were required to undergo a 17-h decompression before they resurfaced.

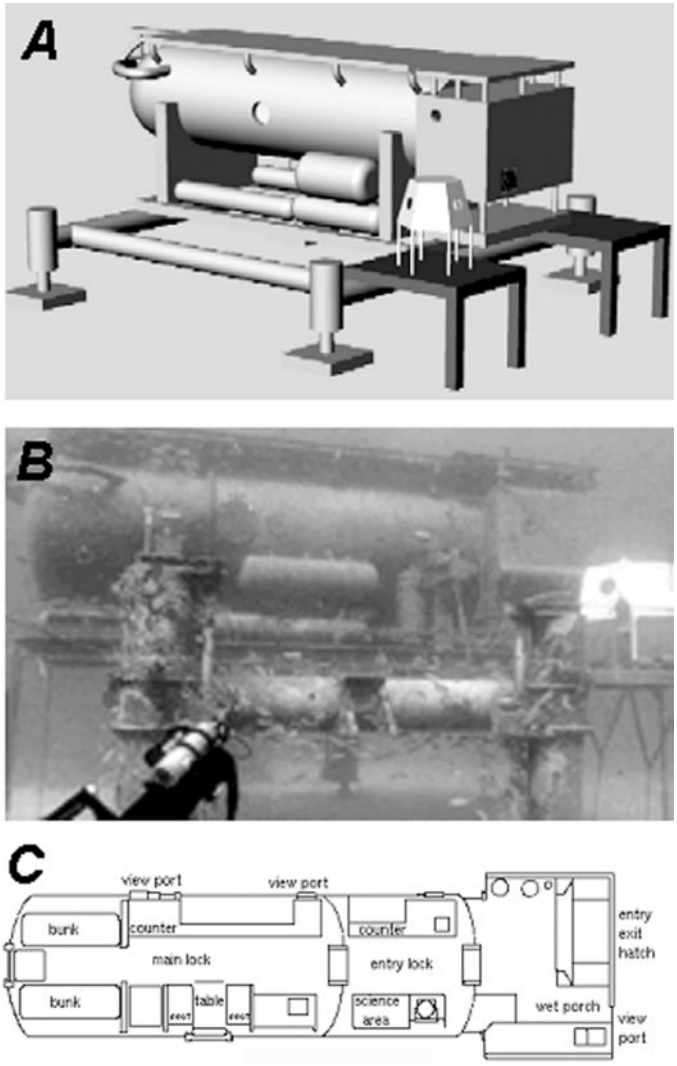


**FIGURE 1** Diagram (*A*), photograph (*B*), and floorplan (*C*) of the NASA Extreme Environment Mission Operations (NEEMO) habitat. The habitat is 14 m long and 4 m in diameter, 19 m below the ocean surface. The pressure inside the habitat is 253 kPa (2.5 atm).

***Subjects.*** This study was approved by the Johnson Space Center Committee for the Protection of Human Subjects. The crew for NEEMO V consisted of 2 women and 4 men. Three of the 6 were astronauts (1 with previous flight experience), 1 was a scientist from the Johnson Space Center, and the remaining 2 were technicians responsible for maintaining the habitat. Subjects were trained on all procedures required for the successful completion of the in-dive sample and data collections. Two of the crew members were trained in phlebotomy techniques, and they subsequently collected all pre- and in-dive blood samples.

The mean age was 35.7 ± 6.6 y (mean ± SD). All subjects were required to pass an Air Force Class III physical examination and were required to have logged a minimum of 25 dives before they participated in the study. Before the dive, the mean body mass was 69.9 ± 17.3 kg. For 4 of the crew members, body fat mass (15.3 ± 2.3 kg), bone mineral content (2.5 ± 0.7 kg), and lean body mass (52.1 ± 14.5 kg) were also recorded.

***Dietary intake.*** Predive dietary intake data were collected from a standard FFQ, based on self-selected diets (10). Space foods, packaged as for flight or provided in bulk, were consumed throughout the dive. In-dive food intakes were recorded using a barcode reader, which recorded subject identification, time and date of entry, and quantity of each item consumed (also allowing for recording of partial food items consumed). A dietary logbook was provided to each crew member, along with a scale, to record any food consumed from non-space food packaging (i.e., those without a barcode label). Dietary training was provided by the research dietitian (B.L.R.) before the mission, and all dietary data were analyzed by the research dietitian. Nutrient calculations were performed using the Nutrition Data System for Research (NDS-R) (11) Version 4.06.34 developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN, Food and Nutrient Database 34 released May, 2003.

***Body mass and body composition determinations.*** On the predive days when body composition was determined, body mass was measured with a calibrated scale; a standard scale was used on all other predive days. For the in-dive determinations, a standard scale was tested in the habitat and was found to function reliably in the high-pressure atmosphere. This scale was used for the remainder of the study.

Before and after the dive, body composition was determined by dual energy X-ray absorptiometry (DEXA fan beam densitometer; Hologic QDR 4500W, Hologic). Whole-body scans were performed before and after the mission for body composition assessment. Because these scans were performed at the Johnson Space Center, the 2 subjects that were not from Houston were unavailable for these data collections.

***Sample collection and processing.*** Blood (25.7 mL) was collected before (dive minus 12 d, designated D-12, and D-1), during (mission day 7, designated MD 7, and MD 12), and after the dive (return plus 0 days, designated R + 0, and R + 7). For 2 of the subjects, the first predive collection was completed at D-5/-4. These values were combined with the D-12 value. Blood collections were performed at the same time each day after an 8-h fast (MD 12 blood collection was after a 6 h fast).

Urine was collected before (D-12, D-11, and D-1 for 4 of the subjects, and on D-5, D-4, and D-1 for 2 of the subjects), during (MD 7, MD 12), and after the dive (R + 0, R + 1, R + 7, and R + 8). D-5 and D-4 data were combined with the D-12 and D-11 data, respectively. Pre- and postdive samples were collected in individual bottles and kept cool until they were processed (<24 h). During the dive, the crew collected voids into either a beaker or a graduated cylinder. The volume of each void was recorded, and a 50-mL aliquot was sent to the surface. All urine and blood samples were kept in a cooler on ice in the habitat before (and during) ascent to the surface. The samples were also kept on ice aboard the boat during its return to shore. Urine pools (24 h) based on void volumes were created, pH was measured,

and aliquots were prepared and frozen for analysis as soon as possible on shore.

If storage of blood samples would alter the results of a test [such as malondialdehyde (MDA), hematocrit, and hemoglobin], the test was run in the laboratory facilities on shore. Aliquots used for other tests remained frozen at −20°C until shipment on dry ice for return to the Johnson Space Center in Houston.

***Biochemical analyses.*** Most analytical determinations were performed by standard commercial techniques as described previously (6). Hemoglobin, hematocrit (calculated), and mean corpuscular volume (MCV) were determined using a Coulter T890 instrument (Beckman Coulter). Serum ferritin and transferrin were analyzed with the Immulite (Diagnostics Products) and Array 360 (Beckman Coulter) instruments, respectively. Transferrin receptors were measured using a commercially available ELISA (Ramco Laboratories). RBC folate was measured using a commercially available radioreceptor assay (Diagnostic Products). Ferritin iron content was determined by inductively-coupled plasma MS (ICP-MS) using a method previously described (6).

Whole-blood ionized calcium and electrolytes were determined using ion-sensitive electrode techniques with a portable analyzer (i-STAT) (6,12). The portable analyzer did not function properly in the underwater laboratory, however, because of the pressure differential. These tests were performed after samples had been brought to the surface.

Urine and serum total calcium concentrations were measured by ICP-MS techniques (13). Serum intact parathyroid hormone was measured by RIA (Nichols Institute Diagnostics). The vitamin D metabolites 25-hydroxyvitamin D (25-OH vitamin D) and 1,25-dihydroxyvitamin D [1,25-$(OH)_2$ vitamin D] were also determined using commercially available RIA kits (DiaSorin). Bone-specific alkaline phosphatase was measured by ELISA (Quidel), and serum osteocalcin was measured by commercial RIA (Biomedical Technologies).

Urine samples were analyzed for collagen crosslinks using commercially available kits [METRA pyridinium (PYD) and deoxypyridinoline (DPD) EIA kits, Quidel; and Osteomark ELISA kit; Ostex International] as previously described (14). Crosslink data were expressed as nmol excretion/d because we showed that this reduces within-subject variability (15).

RBC superoxide dismutase (SOD), glutathione peroxidase (GPX), and serum oxygen-radical absorbance capacity were measured spectrophotometrically using commercially available kits (Randox Laboratories). HPLC techniques (16) were used to determine 8-hydroxy-2′-deoxyguanosine (8OHdG) in urine. Plasma MDA was measured using a commercially available kit (Calbiochem Lipid Peroxidation Assay kit, EMD Biosciences).

Serum total protein, cholesterol, triglycerides, sodium, potassium, chloride, aspartate aminotransferase, alanine aminotransferase, RBC transaminase activity, RBC glutathione reductase activity, and total alkaline phosphatase were analyzed using a Beckman CX7 automated clinical chemistry system (Beckman Coulter). RBC transaminase and glutathione reductase activity assays are functional indicators of vitamin B-6 and riboflavin status, which assess the in vitro activity of the enzymes. Serum albumin and transthyretin were analyzed with the Beckman Appraise and Array 360 instruments, respectively (Beckman Coulter). Urine creatinine was analyzed on the NexCT clinical chemistry system (Alfa Wassermann).

***Statistical analysis.*** Data are reported as means ± SD. Dietary data and biochemical data were analyzed by repeated-measures ANOVA (RM-ANOVA) with a post-hoc Bonferroni test to determine differences among groups. For all of the predive data, a paired *t* test (blood) and RM-ANOVA (urine) were performed to determine baseline differences. If none existed, a mean value was calculated and an RM-ANOVA was performed comparing the predive value to all in- and postdive samples. If there were predive differences, each data point was compared individually using RM-ANOVA. Statistical analyses were performed using SigmaStat (SPSS). Differences were considered significant at $P < 0.05$.

## RESULTS

***Dietary intake.*** An FFQ was used to determine predive dietary intakes for energy (8791 ± 2566 kJ), fat (84.7 ± 24.2 g), protein (83.1 ± 33.0 g), calcium (774 ± 327 mg), and iron (19.7 ± 14.4 mg). During the dive on MD 5, 6, and 11, energy intake was significantly lower than the WHO recommendations (8414 ± 3709, 7493 ± 2738, and 8832 ± 1515 kJ, respectively). In-dive means were also determined for intakes of vitamin D (3.78 ± 5.29 μg), fat (65 ± 18 g), protein (79 ± 27 g), calcium (1015 ± 391 mg), iron (23 ± 9 mg), and water (2697 ± 1184 mL) using the NDS-R database.

***Body mass and composition.*** On MD 7–14, body mass was significantly lower (74.6 ± 15.7, 74.3 ± 15.8, 74.5 ± 15.9, 74.6 ± 15.6, 74.2 ± 15.8, 74.7 ± 15.9, 74.5 ± 15.9, and 74.2 ± 15.8 kg, respectively, $P < 0.05$) than predive mass (76.0 ± 16.1 kg), and on R + 7 it was higher than predive mass (77.2 ± 16.7 kg). On R + 7, body mass (69.4 ± 17.1 kg), body fat (14.9 ± 2.9 kg), bone mineral content (2.5 ± 0.7 kg), and lean body mass (51.9 ± 14.2 kg) did not differ ($n = 4$) from predive values.

***Hematology and general chemistry.*** On R + 0, both hemoglobin and hematocrit were decreased ($P < 0.05$) from their predive and MD 7 values (**Table 1**). On the last postdive collection day (R + 7), serum MCV was lower ($P < 0.05$) than its in-dive (MD 7 and 12) and R + 0 values (Table 1). Also on R + 7, serum iron concentration was significantly lower than in-dive values (MD 7 and MD 12). RBC glutathi-

**TABLE 1**

*Hematologic, iron, and folate status indicators of NEEMO crew divers before, during, and after a 14-d saturation dive*[1]

| | Pre | MD 7 | MD 12 | R + 0 | R + 7 |
|---|---|---|---|---|---|
| Hemoglobin, *g/L* | 136 ± 15$^{a}$ | 138 ± 10$^{a}$ | 137 ± 14$^{ab}$ | 128 ± 13$^{b}$ | 131 ± 13$^{ab}$ |
| Hematocrit | 0.41 ± 0.04$^{a}$ | 0.41 ± 0.03$^{a}$ | 0.40 ± 0.04$^{ab}$ | 0.38 ± 0.04$^{b}$ | 0.40 ± 0.04$^{ab}$ |
| MCV, *fL* | 91 ± 3$^{ab}$ | 92 ± 3$^{a}$ | 92 ± 4$^{a}$ | 92 ± 3$^{a}$ | 90 ± 2$^{b}$ |
| Serum iron, *μmol/L* | 19 ± 7$^{ab}$ | 26 ± 10$^{a}$ | 27 ± 10$^{a}$ | 22 ± 5$^{ab}$ | 12 ± 4$^{b}$ |
| Ferritin iron, *μmol/L* | 0.34 ± 0.15 | 0.48 ± 0.19 | 0.44 ± 0.17 | 0.47 ± 0.17 | 0.32 ± 0.12 |
| Serum ferritin, *μg/L* | 102 ± 63$^{b}$ | 168 ± 82$^{a}$ | 219 ± 98$^{a}$ | 196 ± 92$^{a}$ | 117 ± 70$^{b}$ |
| Ferritin saturation, % | 22.8 ± 9.2 | 18.2 ± 8.7 | 12.0 ± 3.2 | 14.6 ± 3.8 | 18.6 ± 8.5 |
| Transferrin receptors, *mg/L* | 4.8 ± 1.3$^{a}$ | 4.6 ± 1.1$^{ab}$ | 4.3 ± 0.8$^{ab}$ | 3.9 ± 0.9$^{b}$ | 4.1 ± 1.0$^{ab}$ |
| Transferrin, *g/L* | 2.63 ± 0.18 | 2.66 ± 0.29 | 2.65 ± 0.14 | 2.53 ± 0.24 | 2.62 ± 0.18 |
| RBC folate, *nmol/L* | 1705 ± 486 | 1598 ± 575 | 1683 ± 365 | 1445 ± 486 | 1554 ± 429 |

[1] Values are means ± SD, $n = 6$ (except on MD 12, when $n = 5$ for all variables except transferrin receptors). Means in a row without a common letter differ, $P < 0.05$.

## TABLE 2

*General blood chemistry of NEEMO crew divers before, during, and after a 14-d saturation dive*[1]

| | Pre | MD 7 | MD 12 | R + 0 | R + 7 |
|---|---|---|---|---|---|
| Serum sodium, *mmol/L* | 138 ± 2[b] | 141 ± 2[a] | 141 ± 2[a] | 139 ± 1[b] | 141 ± 2[a] |
| Serum potassium, *mmol/L* | 4.2 ± 0.3* | 4.1 ± 0.2 | 4.2 ± 0.7 | 3.9 ± 0.3 | 4.3 ± 0.2 |
| Serum chloride, *mmol/L* | 106 ± 2 | 106 ± 3 | 105 ± 3 | 105 ± 2 | 107 ± 3 |
| Serum creatinine, *μmol/L* | 94 ± 13 | 94 ± 13 | 77 ± 40 | 93 ± 17 | 94 ± 16 |
| Serum triglyceride, *mmol/L* | 1.03 ± 0.34 | 1.07 ± 0.38 | 1.36 ± 0.30 | 1.54 ± 0.55 | 1.72 ± 1.00 |
| Serum RBP,[2] *mg/L* | 67.4 ± 7.2 | 58.8 ± 17.9 | 59.3 ± 11.4 | 55.0 ± 11.2 | 65.3 ± 13.4 |
| RBC GSH reductase activity, % | 13.9 ± 10.0 | 7.2 ± 10.5 | 5.7 ± 5.0 | 6.0 ± 7.6 | 10.4 ± 4.9 |
| RBC transaminase activity, % | 81.6 ± 13.4 | 86.0 ± 15.4 | 79.5 ± 16.6 | 86.7 ± 15.4 | 82.1 ± 13.8 |
| Serum ALT, *U/L* | 18.4 ± 7.0 | 13.8 ± 6.2 | 16.2 ± 4.8 | 14.2 ± 3.5 | 18.8 ± 6.9 |
| Serum AST, *U/L* | 25.9 ± 2.6 | 29.7 ± 4.6 | 29.6 ± 10.6 | 26.2 ± 3.9 | 25.3 ± 5.8 |
| Serum ceruloplasmin, *mg/L* | 430 ± 130[b] | 470 ± 150[ab] | 480 ± 120[ab] | 520 ± 120[a] | 500 ± 140[ab] |
| Serum transthyretin, *mg/L* | 305 ± 58 | 285 ± 24 | 312 ± 22 | 305 ± 38 | 327 ± 59 |
| Serum cholesterol, *mmol/L* | 5.15 ± 0.67 | 5.14 ± 1.10 | 4.26 ± 2.26 | 4.78 ± 0.86 | 5.31 ± 0.91 |
| pH | 7.4 ± 0.02 | 7.4 ± 0.02 | 7.4 ± 0.05 | 7.4 ± 0.02 | 7.4 ± 0.04 |
| Total protein, *g/L* | 68 ± 4 | 70 ± 3 | 70 ± 7 | 70 ± 3 | 69 ± 4 |
| Albumin, *g/L* | 43 ± 3 | 46 ± 2 | 45 ± 3 | 45 ± 3 | 45 ± 3 |
| Leptin, *μg/L* | 5.8 ± 3.5[a*] | 4.2 ± 2.6[ab] | 2.7 ± 1.2[ab] | 2.3 ± 1.1[b] | 5.6 ± 2.4[a] |
| CRP, *mg/L* | 1.8 ± 1.9 | 11.9 ± 18.1 | 12.4 ± 23.3 | 4.7 ± 7.4 | 2.1 ± 2.4 |

[1] Values are means ± SD, $n = 6$ (except on MD 12, when $n = 5$ for all variables except serum RBP). * Value is from D-1 session. Means in a row without a common letter differ, $P < 0.05$.

[2] RBP, retinol binding protein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein. RBC GSH reductase and transaminase activities are functional tests that assess the in vitro activity of the enzymes, and are expressed as % activation after the addition of riboflavin or vitamin B-6 (respectively) to the assay.

one reductase activity tended to be decreased ($P = 0.07$) during the dive (**Table 2**).

On both in-dive days and R + 0, serum ferritin was significantly greater than it was before the dive and on R + 7 (Table 1). Transferrin receptors were significantly reduced on R + 0 (Table 1). Ferritin iron, ferritin saturation, RBC folate, and transferrin did not change throughout the study.

Triglycerides tended to increase after the dive ($P = 0.08$). Serum leptin was elevated the day before diving (D-1) compared with the earlier predive session and levels at R + 0 were significantly lower than those at D-1 and R + 7 (Table 2).

Electrolyte pools also changed in response to conditions during NEEMO V. Sodium and chloride excretions were less ($P < 0.05$) during the dive (MD 7 for chloride and MD 12 for sodium and chloride) and on R + 0 compared with predive (**Table 3**). Urine excretion volume remained constant during the study. Serum sodium concentration was significantly higher during (MD 7 and 12) and after (R + 7) the dive compared with predive and R + 0 ($P < 0.05$, Table 2). Whole blood sodium was also elevated on MD12 compared with predive and R + 0 (data not shown). Serum potassium was elevated on D-1 compared with the earlier predive session. There was no difference in any of the other general blood and urine chemistry indices.

***Calcium and bone metabolism.*** Urinary calcium excretion was significantly greater after the dive (R + 8) than in mid-dive (MD 12) (**Table 4**). Serum total calcium concentration did not change during the study, but blood ionized calcium was increased ($P < 0.05$) on MD 7 and 12 compared with predive (Table 4). Urinary collagen crosslinks (DPD, *n*-telopeptide, and PYD) were unaffected during and after the dive.

## TABLE 3

*General urine chemistry of NEEMO crew divers before, during, and after a 14-d saturation dive*[1]

| | Pre | MD 7 | MD 12 | R + 0 | R + 1 | R + 7 | R + 8 |
|---|---|---|---|---|---|---|---|
| Urine pH | 6.0 ± 0.2 | 6.2 ± 0.4 | 6.3 ± 0.3 | 6.1 ± 0.5 | 6.5 ± 0.5 | 6.2 ± 0.6 | 6.0 ± 0.3 |
| Excretion volume, *mL* | 2155 ± 676 | 1965 ± 1263 | 2414 ± 944 | 2671 ± 1384 | 1666 ± 647 | 2111 ± 1040 | 2624 ± 1944 |
| Sodium, *mmol/d* | 182 ± 21[a] | 120 ± 71[ab] | 106 ± 44[b] | 99 ± 32[b] | 124 ± 42[ab] | 163 ± 31[ab] | 191 ± 58[a] |
| Potassium, *mmol/d* | 62.2 ± 15.7 | 47.3 ± 15.0 | 53.0 ± 18.5 | 46.8 ± 18.2 | 53.8 ± 24.1 | 72.5 ± 25.2 | 64.8 ± 20.7 |
| Chloride, *mmol/d* | 176 ± 16[ac] | 98.5 ± 60.4[b] | 90.2 ± 31.9[b] | 87.7 ± 30.6[b] | 107 ± 24[bc] | 159 ± 25[abc] | 193 ± 69[a] |
| 3MH,[2] *μmol/d* | 245 ± 122 | 242 ± 138 | 222 ± 116 | 230 ± 93 | 270 ± 104 | 271 ± 112 | 269 ± 114 |
| Iodine, *mmol/d* | 2.46 ± 0.91 | 2.12 ± 0.84 | 1.77 ± 0.71 | 2.68 ± 1.13 | 1.80 ± 0.29 | 2.18 ± 0.96 | 2.60 ± 1.39 |
| Mg, *mmol/d* | 3.89 ± 1.05 | 4.34 ± 1.39 | 3.78 ± 1.44 | 4.67 ± 1.67 | 4.09 ± 1.14 | 4.43 ± 0.94 | 5.06 ± 1.60 |
| Phosphate, *mmol/d* | 30 ± 7 | 34 ± 11 | 33 ± 12 | 27 ± 10 | 23 ± 8 | 29 ± 11 | 29 ± 6 |
| Creatinine, *mmol/d* | 16.3 ± 3.7 | 15.2 ± 5.2 | 17.5 ± 5.2 | 16.2 ± 4.0 | 16.2 ± 3.6 | 16.3 ± 4.0 | 16.1 ± 3.5 |

[1] Values are means ± SD, $n = 6$ ($n = 5$ on R + 1). Means in a row without a common letter differ, $P < 0.05$.

[2] 3MH, 3-methylhistidine.

**TABLE 4**

*Calcium and bone markers in NEEMO crew divers before, during, and after a 14-d saturation dive*[1]

| | Pre | MD 7 | MD 12 | R + 0 | R + 1 | R + 7 | R + 8 |
|---|---|---|---|---|---|---|---|
| Serum iPTH,[2] *ng/mL* | 31.1 ± 7.8 | 25.8 ± 9.7 | 24.3 ± 6.9 | 28.8 ± 10.2 | — | 29.6 ± 9.8 | — |
| Serum osteocalcin, *μg/mL* | 9.3 ± 2.1$^{ab}$ | 7.4 ± 1.0$^{b}$ | 7.8 ± 1.5$^{b}$ | 8.4 ± 1.7$^{ab}$ | — | 10.7 ± 2.9$^{a}$ | — |
| Alkaline phosphatase | | | | | | | |
| Serum total, *U/L* | 57.7 ± 22.0 | 61.0 ± 17.7 | 66.6 ± 20.0 | 61.7 ± 22.3 | — | 61.3 ± 21.5 | — |
| Serum BSAP, *U/L* | 21.7 ± 7.8 | 20.7 ± 7.2 | 21.4 ± 8.6 | 21.7 ± 8.1 | — | 19.7 ± 7.5 | — |
| Serum 25-OH vitamin D, *nmol/L* | 92 ± 23 | 86 ± 30 | 81 ± 18 | 86 ± 18 | — | 81 ± 24 | — |
| Serum 1,25-$(OH)_2$ vitamin D, *pmol/L* | 184 ± 42* | 212 ± 105 | 221 ± 81 | 198 ± 108 | — | 201 ± 64 | — |
| Calcium | | | | | | | |
| Serum total Ca, *mmol/L* | 2.56 ± 0.11 | 2.70 ± 0.18 | 2.57 ± 0.15 | 2.57 ± 0.10 | — | 2.67 ± 0.22 | — |
| Ionized Ca, *mmol/L* | 1.18 ± 0.03$^{b}$ | 1.23 ± 0.05$^{a}$ | 1.26 ± 0.02$^{a}$ | 1.21 ± 0.03$^{ab}$ | — | 1.21 ± 0.03$^{ab}$ | — |
| Urine Ca, *mmol/L* | 5.49 ± 0.73$^{ab}$ | 4.83 ± 1.85$^{ab}$ | 3.98 ± 0.91$^{b}$ | 5.13 ± 1.51$^{ab}$ | 4.92 ± 1.21$^{ab}$ | 6.12 ± 2.33$^{ab}$ | 6.82 ± 2.18$^{a}$ |
| DPD, *nmol/d* | 63.9 ± 7.4 | 73.7 ± 24.9 | 70.2 ± 20.2 | 61.6 ± 15.5 | 60.3 ± 8.6 | 66.8 ± 17.3 | 57.0 ± 19.8 |
| NTX, *nmol/d* | 464 ± 235 | 690 ± 384 | 480 ± 287 | 430 ± 208 | 499 ± 271 | 539 ± 363 | 556 ± 240 |
| PYD, *nmol/d* | 256 ± 51 | 307 ± 85 | 292 ± 78 | 297 ± 98 | 224 ± 68 | 285 ± 87 | 260 ± 89 |

[1] Values are means ± SD, $n = 6$ ($n = 5$ on MD 12 for total alkaline phosphatase and $n = 5$ on R + 1 for DPD, NTX, and PYD). Means in a row without a common letter differ, $P < 0.05$. * Values are from D-1 session.

[2] iPTH, intact parathyroid hormone; BSAP, bone-specific alkaline phosphatase; NTX, *n*-telopeptide.

Serum osteocalcin concentration was significantly greater after the dive (R + 7) than on MD 7 and 12. The concentration of other markers of bone formation, including serum total alkaline phosphatase and bone-specific alkaline phosphatase, did not change significantly during the study (Table 4). 1,25-$(OH)_2$ Vitamin D was decreased the day before diving (D-1) compared with the earlier predive measure. Nevertheless, there was no effect of the dive on serum vitamin D metabolites, 25-OH vitamin D, or 1,25-$(OH)_2$ vitamin D (Table 4).

***Antioxidant status.*** When R + 0/1 were combined and R + 7/8 were combined, urinary 8-hydroxy-2′deoxyguanosine [8(OH)dG excretion was higher ($P < 0.05$) in-dive (MD7 and MD12) than predive (**Table 5**). Other markers of antioxidant status and function were altered during and postdive, including whole-blood GPX, SOD, and plasma MDA. GPX was significantly decreased on R + 7 (Table 5). SOD was decreased on MD 7, and this decrease ($P < 0.05$) compared with predive levels continued on MD 12 and R + 7 (levels on R + 0 tended to be lower). Plasma MDA was significantly decreased postdive (R + 0 and R + 7) compared with predive.

## DISCUSSION

Limitations on mission resources (such as time, power, volume, mass) for spaceflight research necessitate the development of Earth-based analogs. The underwater isolation of the NEEMO missions provides one such analog of spaceflight, with obvious similarities but also obvious limitations. The study we report here clearly identifies the underwater laboratory as a valuable analog environment, and the results show that this environment resembles spaceflight in many aspects other than the directly nutritional ones (such as dietary intake). When we have further defined the changes that occurred in crew members, we will be better able to propose, design, and test countermeasures for future missions.

The hematologic findings are striking. They extend those from earlier dive studies and are similar to hematologic changes seen during spaceflight. Reductions in hemoglobin concentration and increases in serum ferritin concentration are consistently observed in deep saturation dives (depths up to 660 m, 3141–6789 kPa) (17–19). These effects were observed after the 14-d shallow saturation dive described here (19 m below sea level, 253 kPa). Reduced hemoglobin con-

**TABLE 5**

*Antioxidants and oxidative damage indicators in NEEMO crew divers after a 14-d saturation dive*[1]

| | Pre | MD 7 | MD 12 | R + 0 | R + 1 | R + 7 | R + 8 |
|---|---|---|---|---|---|---|---|
| 8(OH)dG, *μg/d* | 5.7 ± 1.3 | 7.3 ± 2.3* | 7.4 ± 2.1* | 6.8 ± 2.4 | 5.8 ± 1.4 | 5.8 ± 1.5 | 5.7 ± 1.5 |
| GPX, *U/g hemoglobin* | 63.8 ± 6.4$^{a}$ | 54.1 ± 9.1$^{ab}$ | 58.6 ± 9.7$^{ab}$ | 53.1 ± 9.2$^{ab}$ | — | 48.1 ± 7.2$^{b}$ | — |
| MDA, *μmol/L* | 0.75 ± 0.46$^{a}$ | 0.61 ± 0.22$^{ab}$ | 0.60 ± 0.25$^{ab}$ | 0.26 ± 0.13$^{b}$ | — | 0.20 ± 0.05$^{b}$ | — |
| SOD, *U/g hemoglobin* | 1240 ± 185$^{a}$ | 877 ± 173$^{b}$ | 912 ± 185$^{b}$ | 1029 ± 170$^{ab}$ | — | 997 ± 101$^{b}$ | — |
| TAC,[2] *mmol/L* | 1.12 ± 0.03 | 1.15 ± 0.15 | 1.39 ± 0.47 | 1.06 ± 0.09 | — | 1.12 ± 0.06 | — |

[1] Values are means ± SD, $n = 6$ [$n = 5$ for GPX, MDA, and SOD for MD 12 and $n = 5$ for 8(OH)dG on R + 1]. Means in a row without a common letter differ, $P < 0.05$. * When R + 0/1 and R + 7/8 were combined, in-dive 8(OH)dG was significantly ($P < 0.05$) higher than predive.

[2] TAC, total antioxidant capacity.

centrations suggest that RBC mass was reduced. This could be caused by decreased production of new RBC, as seen in spaceflight (20), or destruction of existing RBC by oxidative damage (17,21).

We also observed increased serum ferritin concentrations during the dive and decreased concentrations of transferrin receptors on R + 0. Such findings would be expected when iron stores and intracellular iron availability are high. It is likely that the increased oxygen availability, induced by the increased atmospheric pressure, contributed to a decreased need for RBC, and iron pools were consequently shifted from hemoglobin to a storage form. This process, termed neocytolysis, has been documented to occur in spaceflight (22,23), as well as in subjects traveling from high to low altitude (24).

Although ferritin iron content did not increase along with the increased serum ferritin concentration, ferritin iron and serum iron both tended to increase during the dive. One possible explanation for the lack of significance of these increases is the small sample size ($n = 6$ for before the dive, MD 7, R + 0, and R + 7; $n = 5$ for MD 12). Another possible explanation is that, as indicated by the increase in serum ferritin concentration, ferritin was being recruited from preexisting stores, and the time course was too short for enrichment of serum ferritin with excess iron to be reflected in the serum concentration of iron. It is also possible that the changes in serum ferritin during the dive were caused by an acute inflammatory response, whose occurrence was indicated by other results. The serum concentration of other acute phase proteins tended to increase during the dive. Although the increases were not significant, the serum concentration of C-reactive protein tended to be greater during the dive than before and after the dive. The large variances prevented these findings from being significant. Again, the very small sample size in this study limits the conclusions that can be drawn. However, other studies suggest that oxidative stress increases during the acute inflammatory phase of many illnesses (25,26), and this was observed in 1 subject before the dive.

Changes in the concentration of antioxidant markers were expected because of the hyperbaric environment. Along with the increased 8(OH)dG excretion during the dive, decreases in GPX and SOD during (SOD) and after (GPX and SOD) the dive suggest that oxidative stress increased. A number of other factors (in addition to the environment) could have contributed to this, including changes in nutrient intake and changes in stress hormones. The significant decrease in MDA concentration suggests that lipid peroxidation was lower during and after the dive than before the dive. This would not support the explanation of increased oxidative damage. The decrease in lipid peroxidation is not easily explained because during the dive, we would have expected it to be accompanied by similar changes in 8(OH)dG and MDA. Predive means of all variables are means of measurements recorded twice before the dive. The concentration of MDA tended to be greater ($P = 0.06$) on the earlier predive session ($1.25 \pm 0.96$ $\mu$mol/L) than on D-1 ($0.26 \pm 0.18$ $\mu$mol/L). When only D-1 was used for comparison (instead of the mean of the 2), the concentration of MDA was greater during the dive than before or after the dive.

During the latter part of the dive (MD 7–14), mean body weights were significantly lower than they were before the dive. During the dive, energy intakes were lower than WHO recommendations. This also consistently occurs during spaceflight (6,27,28) and explains why body weights were concurrently decreased. Serum leptin was measured in these individuals and we found that these concentrations were significantly decreased by the last day (R + 0). Leptin is normally involved in the regulation of food intake and in the maintenance of energy balance, but its role in the decreased energy intake in this study is unknown and warrants further investigation. Other studies have linked decreased leptin concentration with periods of intense exercise, possibly indicative of increased stress or inflammation (29,30). The decreased leptin observed here, consistent with other findings outlined above, may support the occurrence of an acute inflammatory response during the dive.

Despite the increased atmospheric pressure in the habitat, bone formation and resorption did not change measurably during the dive. Although osteocalcin was significantly greater after the dive (R + 7) than during it (MD 7 and 12), other bone formation markers in the serum, including alkaline phosphatase and bone-specific alkaline phosphatase, were unchanged during the study. Bone resorption markers were unchanged during the dive. Concentrations of parathyroid hormone and 25 OH vitamin D tended to decrease, but not significantly. Both of these indices might have become significant during or after a longer mission (due to lack of UV light exposure) or with additional subjects. These findings enhance our recent observations that lower body negative pressure (LBNP) can mitigate disuse-induced bone resorption (31). The current study, one of whole-body positive pressure, suggests that the findings with LBNP may be related more to circulatory changes than to pressure itself. Such suggestions that circulatory influences may affect weightlessness-induced bone loss are not new (32,33).

Many physiologic and nutritional changes that occurred during NEEMO V are also commonly observed during spaceflight. Changes in nutritional status during spaceflight are of critical concern for future long-duration space travel, and spaceflight analogs such as NEEMO V may be increasingly important for investigation of potential countermeasures.

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